However, in both cases, there is the potential for multiple electrostatic interactions to sum and yield potent binding. Our data suggest that pore-block, and acceleration of inactivation, result from distinct molecular actions of the toxin.

700-Pos Board B469
Long Molecular Dynamics Simulations of the Voltage-Gated Sodium Channel, Na_vAb
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The solution of the Na_vAb ion channel crystal structure has provided a first glimpse of the inner workings of the voltage-gated Na^+ channel family, which are central to electrical signaling in the body. We have carried out a set of multi-microsecond molecular dynamics simulations aimed at shedding light on the mechanisms of permeation and selectivity for this unusual channel, with its selectivity filter EEEE locus being more reminiscent of a calcium than a sodium channel. Despite the crystal structure exhibiting a closed pore, these simulations, on the physiological timescale for permeation, have revealed exchanges between accessible multi-ion configurations (with 2-3 ions in the pore) and their coordination by water and protein groups, as well as the competition between Na^+, K^+ and Ca^{2+} ions within the selectivity filter. We observe a critical influence of glutamate protonation on the formation of multiple ion occupancy states, as well as on protein structure and flexibility. Our long simulations have uncovered interesting conformational changes within the voltage sensors and pore lining helices, including simulations that reveal structures consistent with a putative inactivated state. These studies illustrate the valuable contribution long atomistic simulations can make, through observations that can guide targeted computational and experimental studies of ion channel function.

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The First Crystal Structure of a Voltage-Gated Na^+ Channel Predicts a New Determinant of External Access for Hydrophilic Local Anaesthetics
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The frequently used local anaesthetic lidocaine is believed to reach its binding site in the intracellular vestibule of the voltage-gated sodium channel (Na_V)via the cell membrane. QX-222 is a permanently charged, quaternary amine analogue of lidocaine that can access this binding site via a hydrophilic route across the channel protein. This pathway exists in the wild-type heart sodium channel ($Na_V1.5$). In addition, mutations at a site in the upper part of the S6 segment in domain IV have also been shown to open such an external access pathway (EAP; positions 1760 in $rNa_V1.2$, 1575 in $rNa_V1.4$; Ragsdale et al. *Science* 265:1724,Sunami et al. *Mol. Pharmacol.* 59:684).
In the first crystal structure of a Na_V (Na_VAb, Payandeh et al. *Nature* 475:353) a tryptophan (W179, homologous to W1531 in rNav1.4) of the P-loop in domain IV is positioned in close proximity to I202 (homologous to I1575 of rNav1.4). Therefore, we tested the hypothesis that mutations at site W1531 may modulate the EAP.
Whole-cell patch clamp measurements were performed on tsA201 cells transiently transfected with $rNa_V1.4$ constructs. Development of block was assessed by by application of 25 ms pulses to 0 mV at 2 Hz stimulation frequency from a holding potential of −120 mV.
QX-222 blocked currents through W1531A and W1531G by 21 ± 3% and 15 ± 2%, respectively. In both constructs block development was extremely fast (time constants: ~3s and ~2s for W1531A and W1531G), i.e. ~10-20 fold more rapid than I1575A (~40s). Thus, mutations at site 1531 open an access pathway allowing for rapid block by QX-222, as predicted from the crystal structure of Na_VAb.
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Structural Basis for Activation of Voltage-Gated Cation Channels
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The critical role of voltage-gated cation channels (VGCCs) relies on a complex voltage-dependent activation mechanism linking two physiologically relevant channel states, open-activated (AT) and closed-resting (RT). Following the early publication of the x-ray crystal structure of the mammalian Kv1.2 channel in the AT conformation, atomistic models for the RT state of the channel have been proposed. For all of these models, structural analyses demonstrated a consensual explanation of experimental data, thereby highlighting the unambiguous nature of these RT structures. Taken together, these structural studies on Kv1.2 have contributed so far with most of our atomic-level knowledge on the activation mechanism of VGCCs. More recently, the x-ray structure of a prokaryotic voltage-gated sodium channel, NavAb, was resolved in a conformation that was interpreted as representative of the pre-open state of the channel. As one of the possible ancestors of the large family of vertebrate voltage-gated Na^+ and Ca^{++} channels, the appearance of the NavAb structure has provided us with a first, and so far unique, template to extend our knowledge towards other members of the large family of VGCCs. Accordingly, in this contribution, we have considered the well-understood AT and RT structures of Kv1.2, equilibrated in a lipid bilayer, as guide structural models to drive a series of molecular dynamics (MD) simulations aimed at to study the activation process of NavAb. While identifying the reported NavAb structure as an intermediate conformation, not fully-activated, our work has enabled us to determine channel conformations likely related to the RT and AT states of the channel. Overall, the structural results support an activation mechanism highly conserved across the entire family of VGCCs.

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Structure of the Ternary Complex of the C-Terminus of the Voltage-Gated Sodium Channel (Na_V) with Calmodulin (CaM) and Fibroblast Growth Factor Homologous Factor (FHF)
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Voltage-gated sodium channel 1.5 ($Na_V1.5$) is the major sodium channel expressed in heart tissue and is responsible for the initial upstroke of the action potential. Calmodulin (CaM) and fibroblast growth factor homologous factor (FHF) have been reported to regulate the inactivation of $Na_V1.5$ by interacting with the C-terminal domain (CTD) of $Na_V1.5$. Clinical studies also correlate many mutations on the C-terminus of $Na_V1.5$ with arrhythmogenic heart diseases such as long QT syndrome and Brugada syndrome.
We have solved the ternary complex structure of $Na_V1.5$CTD/CaM/FHF2B in the absence of calcium. The calcium-free structure shows the calcium-independent binding of calmodulin to the IQ motif of $Na_V1.5$CTD. Strong interactions between $Na_V1.5$CTD and FHF2B are also observed in this structure. Several disease-causing mutations of $Na_V1.5$CTD are found within the regions interacting with FHF2B and calmodulin. We also identified a critical interaction between $Na_V1.5$CTD and FHF2B that contributes to FHF-subtype specificity. The insight provided by this structure will help us to delineate the regulatory mechanisms of CaM and FHF on the voltage-gated sodium channel.

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Structural Modeling of the Human Nav1.7 Sodium Channel Pore
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Voltage-gated sodium channels expressed in peripheral sensory neurons, play an important role in pain signaling. The human isoform hNav1.7 is a key target for the development of new analgesics for pain treatment. However, high-resolution structures of mammalian voltage-gated sodium channels that could be useful for rational drug design are not available. X-ray structures of bacterial voltage-gated sodium channels, NavAb and NavRh, have been recently been solved and serve as useful templates for homology modeling of mammalian voltage-gated sodium channels. Notably, both NavAb and NavRh structures show asymmetric dimer-of-dimers configuration of the pore-forming domain. We generated homology/de novo models of the asymmetric Nav1.7 pore-forming domain using a Rosetta-membrane homology modeling method and the NavAb structure (pdb id: 4DXW) as a template. Multiple sequence alignments of the second pore helix region between hNav1.7 and NavAb have been explored and compared with experimental data concerning side-chain orientation and ligand binding. Docking of tetrodotoxin, u-conotoxin-KIIIA, and the local anesthetic lidocaine to the pore-forming domain of hNav1.7 using Rosetta-Dock and molecular dynamics simulations were performed to test the ability of structural models to fit available experimental data. Structural models of the pore-forming domain of hNav1.7 may be useful for design of analgesics targeting human voltage-gated sodium channels.

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Coupling of Charge Displacement to Na Current Activation is Impaired for DIIS4 Mutations in HypoPP
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